

EpiXpanded Panel A Targeted Test for Genetic Causes of Epilepsy Using a Trio Approach

OVERVIEW

Epilepsy is a clinically and genetically heterogeneous group of genetic disorders; therefore, it can be challenging to predict the disease-causing gene in a patient with epilepsy based on clinical features alone. It is often necessary to perform testing of multiple genes (concurrently or as reflex tests) to identify the underlying genetic cause of epilepsy in an individual. Moreover, new genes are being discovered regularly, making it challenging for clinical laboratories to keep traditional testing panels updated. Additionally, interpretation of the clinical significance of variants in these newly discovered genes is often difficult in the absence of parental testing to clarify which variants are *de novo* or inherited.

The EpiXpanded Panel uses a trio approach that includes concurrent analysis of the affected proband and both parents, which increases the likelihood of identifying a definitive genetic explanation for epilepsy. Depending on the family structure, family history of seizures, and the availability of both parents, other family members of the affected individual may be evaluated in conjunction with the proband. Please contact GeneDx for prior approval when both parents are not available to submit samples for the EpiXpanded Panel. The EpiXpanded Panel is based on whole exome capture (WEC), NextGeneration sequencing (NGS), and targeted analysis of a comprehensive list of genes currently associated with epilepsy. The design of the panel allows for a comprehensive, dynamic gene list that is updated regularly to ensure inclusion of genes recently associated with epilepsy..

INHERITANCE PATTERN/GENETICS

Epilepsy can be caused by genetic disorders, metabolic diseases, trauma, infection, and structural brain abnormalities, although the cause is not known in many cases. A genetic etiology underlies epilepsy in more than 40% of individuals with seizures.¹ The inheritance pattern can be autosomal dominant, autosomal recessive, or X-linked. Recently, multiple studies have reported a high frequency of *de novo* mutations in epileptic encephalopathies, including infantile spasms and Lennox-Gastaut syndrome, highlighting the importance of a trio approach including the affected proband and both parents when performing epilepsy genetic testing.^{2,3,4,5,6} Mutations in a single gene may be associated with different types of seizures (clinical heterogeneity), and conversely, mutations in different genes can cause the same epilepsy phenotype (genetic heterogeneity). In some cases, confirmation of the molecular genetic cause of epilepsy may have implications for treatment and management.

TEST METHODS

Using genomic DNA from the submitted specimen(s), the exonic regions and flanking splice junctions of the genome were captured using a proprietary system developed by GeneDx and sequenced by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100bp or greater paired-end reads. Reads are aligned to human genome build GRCh37/UCSC hg19 and analyzed for sequence variants in targeted genes using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing or another appropriate method is used to confirm all potentially pathogenic variants identified in the proband and relative samples, if submitted. Sequence and copy number alterations are reported according to the Human Genome Variation Society (HGVS) and International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. A list of additional variants not included in the report is available upon request.

Please note that while the EpiXpanded panel captures and sequences the whole exome, analysis is targeted to the specific phenotype-driven gene list for the EpiXpanded panel. The EpiXpanded Panel gene list includes more

than 1300 genes. The list was developed by searching for genes associated with the phenotypes “Seizures”, “Epilepsy”, and “Epileptic Encephalopathy” in multiple sources, including OMIM, HGMD, and Human Phenotype Ontology (HPO) terms categorized within the “Seizure” branch. Additionally, genes were added to the list using GeneDx data from clinical whole exome sequencing done on patients with epilepsy and from emerging literature about new genes causing epilepsy. The gene list is systematically updated at least quarterly. The current gene list is available on our website: (<http://www.genedx.com/testcatalog/genetic-testing-for-neurological-disorders/>)

RESULT REPORTING

The EpiXpanded Panel is performed on the proband and the parental samples (and/or additional relatives) when submitted together for analysis. A single EpiXpanded report will be issued on the affected proband in the family. A separate report will not be issued for unaffected parents or other family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results from the affected individual. If additional reports are requested for other affected family members, additional fees will apply.

The report that is issued for the affected individual will include reportable variants in genes that have been previously associated with epilepsy in the published or emerging literature. Pathogenic/likely pathogenic variants in genes responsible for the phenotype of the patient will be reported; however, because this is a phenotype-driven test of a large number of genes, variants of uncertain significance (VUS) are not routinely reported, only at our discretion. Variants that are considered to be benign or likely benign will not be reported. As the EpiXpanded Panel includes over 1300 genes, the report will not include a comprehensive list of all observed variants. If desired, clinicians can request a separate file containing a list of identified variants that will be provided upon completion of testing.

In rare instances, this test may reveal a pathogenic variant that is not directly related to the test indication. For example, pathogenic variants in genes associated with an increased risk for cancer, cardiac abnormalities, or metabolic defects could be identified. In the event that an incidental finding is identified, this information will be disclosed to the ordering health care provider if it is likely to impact medical care. The absence of reportable incidental findings for any particular gene does not rule out the possibility of pathogenic variants in that gene.

TEST SENSITIVITY

The clinical sensitivity of the EpiXpanded test depends in part on the patient’s clinical phenotype. Previous whole exome sequencing studies have reported identification of a definitive pathogenic mutation in 37-39% of individuals who have epilepsy in association with developmental delay or other neurodevelopmental disorders if testing is done using a trio approach comparing data from the affected proband with both unaffected parents (Lee et al., 2014; McKnight et al., 2015). The sensitivity of this test is expected to be comparable to whole exome sequencing since it uses a trio approach to test a comprehensive list of genes previously associated with epilepsy. The clinical sensitivity is expected to be significantly lower for singleton testing when only the affected proband is tested.

The average coverage of all genes on the panel is greater than 98% at 10X (with a depth of 10 or more reads). Several genes with a high clinical sensitivity have an average coverage of less than 90% coverage at 10X, including ARX, FOXP1, EPM2A, and SLC6A8. The coverage of each gene on the panel for a specific patient may vary, and the actual coverage for each gene is included in the final report.

LIMITATIONS

Some types of genetic disorders, such as those due to nucleotide repeat expansion/contraction, abnormal DNA methylation, and other mechanisms may not be detectable with this test. Additionally, small sections of a few individual genes have inherent sequence properties that yield suboptimal data and mutations in those regions may not be reliably detected. Specifically, the CGG repeat expansions in FMR1 causing fragile X syndrome, the polyalanine repeat expansions in ARX, the common dodecamer repeat expansion in CSTB associated with

Unverricht-Lundborg disease, and abnormal methylation of UBE3A causing Angelman syndrome would not be detectable by this EpiXpanded Panel.

The scientific knowledge available about the function of all genes in the human genome is incomplete at this time. It is possible that the EpiXpanded Panel may identify the presence of a variant in the sequence of an affected individual, but that we will not recognize it as the cause of their disease due to insufficient knowledge about the gene and its function. Even if the EpiXpanded Panel identifies the underlying genetic cause of a disorder in an affected individual, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

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